6

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2000-270890
(43)Date of publication of application : 03.10.2000

---

(51)Int.Cl. C12P 19/14
A23K 1/16
//(C12P 19/14
C12R 1:685)

---

(21)Application number :	11-077602	(71)Applicant :	UNITIKA LTD
(22)Date of filing :	23.03.1999	(72)Inventor :	TANAKA JUNYA YOSHIKAWA GENICHI

---

(54) PRODUCTION OF MANNOSE OR FODDER INCLUDING MANNOSE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a production method capable of easily and inexpensively producing mannose and production method for producing fodder including mannose.

SOLUTION: Mannose is produced by incubating a microorganism having mannan decomposing enzyme productivity in a medium including mannan to express a mannan decomposing enzyme, controlling the temperature of the cultured product and separating mannose from the medium by the action of the expressed mannan decomposing enzyme.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

Copyright (C); 1998,2003 Japan Patent Office

* NOTICES *

Japan Patent Office is not responsible for any
damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the manufacture approach of a mannose or mannose content feed.
[0002]
[Description of the Prior Art] Are added in order to prevent infection of harmful bacteria to feed, or a mannose is use for food, or is various as drugs synthetic powder, such as a mannitol, etc., and is used.
[0003] The approach of a mannose making a molybdic acid a catalyst and manufacturing from a glucose and the approach of manufacturing by making mannose isomerase act on fructose are learned conventionally. Moreover, it is manufactured by understanding the galactomannan contained in the glucomannan contained in wood, konnyaku, etc. Cyamoposis Gum, etc. by acidolysis or the enzyme.
[0004] By the approach of making a molybdic acid a catalyst among these approaches, since actuation is a complicat top and an organic synthesis method, there is a trouble that it is difficult from a viewpoint of safety to use the compound mannose for the application of food etc. Moreover, the present condition is not said to be the sufficient manufacture approach from a viewpoint of there being a fault the yield of a mannose of what is using cheap fructose the raw material by the approach on which mannose isomerase's is made to act being low, and manufacturing a mannose cheaply, either. By the approach of on the other hand carrying out acidolysis of the mannan, it is a big troub when the high cost produced by the characteristic reaction condition manufactures a mannose cheaply, and since production of a mannan dialytic ferment required for manufacture and the enzyme reaction of mannose isolation are performed by the separate system, the approach of understanding a mannan by the enzyme has the room of the furthe improvement in the point of the cost accompanying it.
[0005]
[Problem(s) to be Solved by the Invention] This invention aims at offering the manufacture approach of the mannose which can manufacture a mannose more cheaply and easily, or mannose content feed.
[0006]
[Means for Solving the Problem] In order to solve said technical problem, wholeheartedly, as a result of examination by cultivating a microorganism by the culture medium containing a mannan, this invention persons are only temperature control and came to complete a header and this invention for the ability of a mannose to be manufacture
[0007] That is, after the 1st invention cultivates the microorganism which has mannan dialytic ferment production ability by the culture medium containing a mannan and makes a mannan dialytic ferment discover, it makes a summ the manufacture approach of the mannose characterized by controlling the temperature of a culture and separating a mannose from the mannan in a culture medium according to an operation of the mannan dialytic ferment made to discover. Moreover, after cultivating the microorganism which has mannan dialytic ferment production ability by the culture medium containing a mannan and making a mannan dialytic ferment discover, the 2nd invention controls the temperature of a culture, separates a mannose from the mannan in a culture medium according to an operation of the mannan dialytic ferment made to discover, and makes a summary the manufacture approach of the mannose content feed characterized by to dry the obtained processing object.
[0008]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. As a microorganism which has the mannan dialytic ferment production ability used for this invention what will be limited especially if it is the microorganism which produces the enzyme which decomposes mannans, such as mannanase (mannase) and

mannosidase, and separates a mannose -- not but a Bacillus subtilis (Bacillus subcillus) and mold (an Aspergillus AKYURE ray wardrobe --) Aspergillus AWAMORI, Aspergillus nigre, Aspergillus USAMI, FUMIKORA in SORENSU, TORIKODERURA hull JIANUMU, TORIKODERURA KONINGI, TORIKODERURA RONGIBURAKIATSUMU, TORIKODERURA BIRIDE, a basidiomycete (COL CHIKIUMU, PIKUNOPORUSU KOKKINEUMU), etc. are mentioned. Also in these microorganisms, since the bacillus of an Aspergillus has high mannan dialytic ferment production ability, it is desirable, and especially its Aspergillus nigre (IFO6661, IFO4417, IFO31012, IFO8541, IFO31125) is desirable.

[0009] Moreover, as a culture medium used for culture of such a microorganism and the isolation reaction of a mannose, especially if a mannan is contained, it is not limited, and it does not need to be refined especially as a mannan. As a mannan, the copra meal which is a coconut oil extract residue grinding object, a chinese yam mannan, yam mannan, locust bean gum, soy bean full, TAMUSONGAMU, Cyamoposis Gum, a konnyaku potato, etc. are mentioned concretely. The mannan in a culture medium serves as a substrate of a reaction as a carbon source in the culture (enzyme manufacture) phase of a microorganism in the phase of the isolation reaction of a mannose.

[0010] As a component of others in a culture medium, mineral salt (sulfates, such as iron, magnesium, calcium, and potassium, phosphate, hydrochloride, etc.) besides a nitrogen source (sources of organic nitrogen, such as a yeast extract, a peptone, a meat extract, corn steep liquor, and soybean cake; sources of inorganic nitrogen, such as an ammonium sulfate, a urea, and an ammonium nitrate) etc. may be added. moreover, a culture medium -- a liquid and solid-state -- you may be whichever. As a content of the mannan in a culture medium, 0.2 - 80 % of the weight is desirable, and 1 - 60 % of the weight is especially desirable.

[0011] In this invention, by inoculating the microorganism which has mannan dialytic ferment production ability in such a culture medium, and cultivating aerobically near the optimum growth temperature of a microorganism first, it making a mannan dialytic ferment discover in a culture medium, next setting up culture temperature near the optimu temperature of a mannan dialytic ferment, and a mannose is separated from a mannan.

[0012] It is desirable that it is near the optimum temperature of the used microorganism as temperature at the time of making an enzyme discover, and 20-40 degrees C is desirable. Moreover, as culture time amount, 24 - 96 hours is desirable. Moreover, as temperature at the time of separating a mannose, it is desirable that it is the operation optima temperature of an enzyme, it is the temperature to which an enzyme does not deactivate and considering as the temperature which controls growth of a microorganism is still more desirable. Specifically, 45-90-degree C 50-80-degree C 65-70 degrees C are still more preferably good preferably. Although it depends for reaction time on the amount of enzymes discovered from the fungus body, it is desirable on an activity to usually set up in 3 - 24 hours.

[0013] Moreover, in case it changes to a temperature setup of an enzyme reaction, when adding an acid for pH adjustment and a solid medium are being used, it is also possible, in order to increase the amount of generation of a mannose to add water. Thus, according to this invention, a mannose can be manufactured even if it does not do an activity special besides controlling the temperature of a culture. When a copra meal is used especially as a mannan, even if it does not add other components especially in a culture medium, it is possible to use as a solid medium for mannose manufacture by making a moisture content into 40 - 70 % of the weight, for example, and it is possible to manufacture a mannose only by controlling temperature after adding water and a microorganism at copra meals.

[0014] Thus, the obtained mannose can be refined using the various chromatographies which used activated carbon a ion exchange resin further. Moreover, it can be made to crystallize by making it the shape of powder by spray drying or adding ethanol.

[0015] Moreover, in this invention, mannose content feed can be manufactured by drying the processing object containing the mannose obtained by doing in this way using a vacuum dryer, a vacuum churning drier, a drum dryer, band dryer, etc. In this case, in order to hold down the cost of desiccation, it is desirable to use a solid medium as a culture medium. Also in order to suppress growth of saprophytic bacteria and not to make a mannose decompose further as temperature in the case of desiccation, 50-100-degree C 70-90 degrees C are preferably good.

[0016]

[Example] Hereafter, an example explains this invention concretely.

Inoculation of Aspergillus niger (IFO 6661) was carried out to the solid medium which added water 100mL to exam 1 copra meal 100g (10 % of the weight of fat, 7.2 % of the weight of moisture), and stationary culture was carried ou to it at 37 degrees C for 48 hours. Subsequently, culture temperature was raised to 65 degrees C, and culture was continued for 16 hours. The culture was suspended in the water of 1L after culture termination, and clear solution 1L containing a mannose was obtained by filtering a supernatant. The high-speed liquid column chromatography analyz the sugar in this solution. the column for analysis -- the Bio-Rad make -- Aminex HPX-87P were used. Column temperature was made into 85 degrees C and rate-of-flow 0.6 mL/min, and performed elution with water. Detection sugar calculated the content of a mannose from the quantum value of a reference standard using the suggestion refractometer. As a result of analyzing the solution after the above-mentioned reaction, the 22g mannose was being accumulated into 1L.

[0017] Subsequently, after degreasing this solution with ethyl ether, it dipped in an anion exchange resin (Dowex SA by the Muromachi chemistry company, OH-mold, bed volume 50mL), cation exchange resin (Dowex HCRW2 made from the Muromachi chemistry, H+ mold, bed volume 50mL), and activated carbon (the Mitsubishi Chemical diamo hope S80, bed volume 50mL) in this sequence, and the honeydew containing a mannose was collected. The collected honeydew was condensed by the evaporator until it became Brix 70, and the honeydew containing a mannose was obtained. The purity of the mannose in this honeydew was 87% (except for moisture).

[0018] Inoculation of Aspergillus niger (IFO 6661) was carried out to the solid medium which added 100ml of water example 2 copra meal 100g (10 % of the weight of fat, 7.2 % of the weight of moisture), and the processing object which contains a mannose like an example 1 was obtained. The vacuum drying of the 90 degrees C of this processin object was carried out for 5 hours with the vacuum dryer (the product made from Yamato, Vaccum Drying Oven DP32), and mannose content feed of about 7.0% of moisture contents was obtained.

[0019] Inoculation of Aspergillus niger (IFO8541) was carried out to liquid-medium (2 % of the weight [of locust b gum], KH2PO4 1%, MgSO4.7H2O0.5%, 0.2% [of yeast extracts], corn-steep-liquor 0.5%, pH5.5) 500mL containi example 3 locust bean gum, and 120rpm performed shaking culture at 30 degrees C for 48 hours. Subsequently, cult temperature was raised to 65 degrees C, and culture was continued for 16 hours. For 15 minutes was performed at 0 degree C after reaction termination, centrifugal separation was performed by 1200rpm, and the culture supernatant w separated. By filtering the obtained culture supernatant, clear solution 475mL containing a mannose was obtained. T high-speed liquid column chromatography analyzed the sugar in this solution. the column for analysis -- the Bio-Rad make -- Aminex HPX-87P were used. Column temperature was made into 85 degrees C and rate-of-flow 0.6 mL/min and performed elution with water. Detection of sugar calculated the content of a mannose from the quantum value of reference standard using the suggestion refractometer. As a result of analyzing the solution after the above-mentione reaction, the 4.1g mannose was being accumulated into 1L.

[0020] Subsequently, after degreasing this solution with ethyl ether, it dipped in an anion exchange resin (Dowex SA by the Muromachi chemistry company, OH-mold, bed volume 50mL), cation exchange resin (Dowex HCRW2 made from the Muromachi chemistry, H+ mold, bed volume 50mL), and activated carbon (the Mitsubishi Chemical diamo hope S80, bed volume 50mL) in this sequence, and the honeydew containing a mannose was collected. After condensing the collected honeydew by the evaporator until it became Brix 70, heat ethanol was added so that it migh become last concentration 85 capacity %, a small amount of D-mannose was added, and it was left at 4 degrees C. T 2.8g crystal mannose was obtained by filtering this solution. The melting point of the obtained mannose was 131.5-132.5 degrees C.

[0021] Inoculation of Aspergillus niger (IFO 4417) was carried out to liquid-medium (2 % of the weight [of Cyamoposis Gum], KH2PO4 1 % of the weight, MgSO4.7H2O0.5%, 0.2% [of yeast extracts], corn-steep-liquor 0.5%, pH5.5) 500mL containing example 4 Cyamoposis Gum, and 120rpm performed shaking culture at 30 degrees for 48 hours. Subsequently, culture temperature was raised to 65 degrees C, and culture was continued for 16 hours. For 15 minutes was performed at 0 degree C after culture termination, centrifugal separation was performed by 1200rpm, and the culture supernatant was separated. By filtering the obtained culture supernatant, clear solution 490 containing a mannose was obtained. The high-speed liquid column chromatography analyzed the sugar in this solutio the column for analysis -- the Bio-Rad make -- Aminex HPX-87P were used. Column temperature was made into 85 degrees C and rate-of-flow 0.6 mL/min, and performed elution with water. Detection of sugar calculated the content a mannose from the quantum value of a reference standard using the suggestion refractometer. As a result of analyzi the solution after the above-mentioned reaction, the 3.1g mannose was being accumulated into 1L.

[0022] Subsequently, after degreasing this solution with ethyl ether, it dipped in an anion exchange resin (Dowex SA by the Muromachi chemistry company, OH-mold, bed volume 50mL), cation exchange resin (Dowex HCRW2 made from the Muromachi chemistry, H+ mold, bed volume 50mL), and activated carbon (the Mitsubishi Chemical diamo hope S80, bed volume 50mL) in this sequence, and the honeydew containing a mannose was collected. The collected honeydew was condensed by the evaporator until it became Brix 70, and the honeydew containing a mannose was obtained. The purity of the mannose in this honeydew was 89% (except for moisture).

[0023]

[Effect of the Invention] According to this invention, it becomes possible to perform from enzyme production to mannose production only by temperature control, and a mannose and mannose content feed can be manufactured eas and cheaply conventionally.

[Translation done.]